Trends of Plasmodium vivax and Plasmodium falciparum infection in Aligarh

DISSERTATION

Submitted in partial fulfilment of the requirements for the Degree of

MASTER OF PHILOSOPHY

IN

ZOOLOGY

BY

A. MEHRUNNISA

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Certificate

This is to certify that the dissertation ***"Trends of Plasmodium vivax and Plasmodium falciparum infection in Aligarh"*** embodied the original research work carried out by **Miss A. Mehrunnisa**, in the section of Parasitology, Department of Zoology, A.M.U., Aligarh, under my supervision and guidance. I have permitted her to submit it towards the partial fulfilment of the requirement for the degree of Master of Philosophy in Zoology.

Wajihullah

Dr. Wajihullah
Lecturer
Section of Parasitology.

CONTENTS

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A Mehrunnisa

A. MEHRUNNISA

INTRODUCTION

Malaria is essentially a disease of the poor countries and included under 'Tropical diseases' by WHO, Geneva. Of the estimated 500 million episodes and 2.7 million deaths from malaria every year, over 90 percent occur in the African sub-continent (WHO, 1996). The disease is endemic in about 91 countries with many pockets of transmission in countries like India, Brazil, Afghanistan, Sri Lanka, Thailand, Indonesia, Vietnam, Combodia and China and takes lives of millions of people every year globally. Malaria was almost eliminated from India in early 1960s through extensive use of insecticides and antimalarials and number of cases came down to 0.1 million from 75 million. Resurgence of malaria occurred in 1970s because of technical and financial problems (Sharma, 1984 & Sharma and Mehrotra, 1986). In 1990's malaria re-emerged because of insecticide resistance in vector and drug resistance in the parasite, killing thousands of people.

More than 100 *Plasmodium* species have been described causing malaria in a wide range of vertebrates and exhibiting narrowly defined host-specificity (Garnham, 1980). Among 100 species only four infect man which are *Plasmodium vivax*,

Plasmodium falciparum, *Plasmodium malariae* and *Plasmodium ovale*. In India we have *Plasmodium vivax* and *Plasmodium falciparum* causing benign and malignant tertian malaria. In India 60-65% of the malaria infections are reported to be due to *Plasmodium vivax* and 30-35% due to *Plasmodium falciparum* (Adak *et al.* 1998). Of all species of human plasmodia, *Plasmodium falciparum* is highly pathogenic causing malignant tertian malaria as a result of which lot of morbidity and mortality takes place. This parasite shows resistance against routine curative drugs such as chloroquine, amodiaquine, metakelfin, fansidar and mefloquine in endemic areas and posing serious problems in cure of this disease.

An increasing number of malaria epidemics has been documented throughout the world. A changing scenario of malaria is observed and it is found that malaria had been changing its clinical profile. The classic paroxysm is evident in only 40% cases of *Plasmodium falciparum* and 47% *Plasmodium vivax*. On the other hand in rest of the cases continuous or remittent type of fever has been observed. Association of jaundice along with hepatomegaly has also been observed in *Plasmodium falciparum* and *Plasmodium vivax* infections. Splenomegaly along with respiratory distress oligurea with impaired renal function and convulsion or coma has also been observed (Hazra *et al.* 1998).

Available informations indicated that *Plasmodium falciparum* has given rise to formidable drug resistant strain in Asia. The problem of chloroquine resistance in *Plasmodium falciparum* is widespread and now it is leading towards multiple drug resistance against all major antimalarials. Sharma (1999) indicated that in high transmission areas in India there are about 30% cases which showed some level of drug resistance against standard dosage of curative drugs. Follow up studies showed relapses in *Plasmodium vivax* infections. Now reports are coming up regarding tolerance of *Plasmodium vivax* against standard dosage of therapeutic and antirelapse drugs (chloroquine and primaquine) and showing complications somewhat similar to that of *falciparum* malaria. In February 1999 KEM hospital in Mumbai recorded first case of *vivax* malaria showing resistance against routine curative drugs.

Keeping above facts in view present study was conducted to find out proportion of *Plasmodium vivax* and *Plasmodium falciparum* infections and their relapsing and resistance patterns in Aligarh.

HISTORICAL REVIEW

The malaria parasite have been with us since the dawn of time. They probably originated in Africa. Fossils of mosquitoes up to 30 million years old show that the vector for malaria was present well before the earliest history. The specific fever caused by malaria parasites known as 'Agues' received the Italian name 'Malaria', since it was then widely believed that their cause was related to the foul air common near marshy areas. The most important event in the history of malaria took place towards the end of 19th century when the workers in the field of bacteriology and pathology were discovering the cause of infectious disease, observing the morbid change in the organs and tissues.

In 1880 Laveran first saw and described malaria parasite in the blood cell of man. Celli and Marchiafava (1889-90) in Italy described *Plasmodium falciparum*. Romanowsky (1890-91) developed a new method of staining malaria parasites in blood film. Manson put forward the theory of malaria transmission from man to man by mosquito in 1894. Ross (1897) discovered pigmented cyst on the stomach wall of an anopheles mosquito (probably *An. Stephensi*) in India. In (1898) Grassi *et al.* described the cycle of

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human malaria parasite in anopheles mosquitoes. Patrick manson in 1900 proved the theory of mosquito transmission. In 1922 Stephen identified and described *Plasmodium ovale*. James in 1931 reviewed Grassi's theory and suggested that the sporozoites soon after entering the body, invades reticulo-endothelial cells or cells lining the capillaries of blood vessels. Short *et al.* (1948-49) described pre-erythrocytic form of *Plasmodium vivax* and *Plasmodium falciparum* in human liver. In 1952 Jeffery *et al.* demonstrated a 3-day old pre-erythrocytic schizont of *Plasmodium falciparum* in human liver. Garnham *et al.* 1954 discovered schizogony of *Plasmodium ovale*.

Epidemiological studies conducted in Gambella, southern Ethiopia, Thailand and India (Nigatu *et al.* 1992, Thimasank *et al.* 1995 and Sharma, 1996) indicate that the most probable contributing factors for malaria transmission pattern were the rehabilitation and resettlement programmes and agricultural activities undertaken in these countries during past ten years that may have brought changes in socio-economic situation and environmental factors. Most of the work in the field of malaria are being done on *Plasmodium falciparum* causing malignant infection and *Plasmodium vivax* which cause benign infection. Lot of mortality takes place because of *Plasmodium falciparum* infection each year

and therefore much attention is paid to this parasite. It is found that *Plasmodium falciparum* infection is emerging in endemic areas (Mandal *et al.* 1998). A competition is going on between *Plasmodium vivax* and *Plasmodium falciparum* to gain upperhand as a result of which a changed scenario of malaria transmission is observed. Unusual acute and chronic complications of malaria were recorded in Delhi by Mehta *et al.* 1996 with manifestations of tachypnoea and pulmonary oedema and shock due to multiple organ dysfunction syndrome. Other features were meningitis, renal failure, hepato-renal syndrome and neck stiffness etc.

Most of the reports and cross checking in different parts of the world indicate that *Plasmodium falciparum* has developed resistance against standard dosage of curative drugs. As far drug resistance is concerned, *Plasmodium falciparum* showed resistance against chloroquine, amodiaquine, metakelfin, fansidar and mefloquine Resistance in *Plasmodium falciparum* to amodiaquine and quinine was reported in Madang province of Papua New Guinea by Alyaman *et al.* (1996). Multiple drug resistance was observed in *falciparum* malaria by McGready *et al.* between 1991-96 in pregnant women living in western border of Thailand. They were treated with either mefloquine, quinine or both. Out of those treated with mefloquine 6% failed to clear parasitaemia by day 7 and 28% by day 24.

Therapeutic effects of chloroquine in combination with quinine in uncomplicated *falciparum* malaria were studied in 50 Thaimen by Vanjanantas *et al.* 1996. They treated all the patients for 7 days with quinine (10 mg base/ kg, every 8 hourly). A group of patients selected randomly was given oral tetracycline (4 mg/kg, 4 times daily) for the same period while patients in other group were given chloroquine for the first three days. Overall clearance time in the chloroquine or tetracycline group were not significantly different. Recrudescence (R-1 level) were higher in chloroquine group than in tetracycline. Survey conducted during last decade showed that *Plasmodium falciparum* has become resistant to chloroquine in Pakistan and Afghan refugees (Shah *et al.* 1997). A repeated in vivo survey indicated that prescription of chloroquine can lead to 15% increase in the frequency of resistance in a single year. The therapeutic efficacy of chloroquine was assessed by Mharakarwa *et al.* 1997 who reported resistance in 52% patients following chloroquine treatment. In 64 cases of uncomplicated *falciparum* malaria in Zimbabwe they noticed a modest reduction in a sexual parasite density and clinical symptom. However, there was an appreciable therapeutic failure of chloroquine which has declining clinical value as the first line of presumptive treatment for uncomplicated malaria. It is therefore recommended in Zimbabwe

that the second line antimalarial sulphadoxine – pyrimethamine should be disrtibuted to health centre level for patients suffering from malaria. Bojang *et al.* (1998) noticed failure of fansidar and chloroquine in Gambian children in India. Ghosh *et al.* (1992) reported that 58.3% *Plasmodium falciparum* cases did not respond to single dose of chloroquine (10 mg base/kg) in vivo test. With standard dose (25 mg base/kg) 31.2% cases showed resistance i.e. RI (5.6%), RII (9.4%) and RIII (6.2%) levels. Garg *et al.* (1996) treated 17 uncomplicated *falciparum* malaria patients resistant to chloroquine with 3 tablets of sulphadoxine – pyrimethamine. Out of 17, 14 patients responded and were sensitive while 3 patients showed RII grade resistance to sulphadoxine pyrimethamine. These 3 patients then responded to a 7 day course of quinine + doxycycline. Hazra *et al.* (1998) studied 60 cases of *Plasmodium falciparum* and 165 cases of *Plasmodium vivax* clinically. It was observed that malaria had been changing its clinical profile. The classic paroxysm was evident only in 40% cases of *Plasmodium falciparum* and 47.27% of *Plasmodium vivax* malaria. On the other hand remittent type of fever had been observed in 40% and 27.2% cases of *Plasmodium falciparum* and *Plasmodium vivax*, respectively. Absence of classic paroxysm of fever, in association with splenomegaly was reported in 80% and 63.63% of these

infections in conjunction with nausea and / or vomiting leading to clinical mimicry with infective hepatitis. Splenomegaly which has been described as cardial feature of malaria was observed in 40% cases with *Plasmodium falciparum* and 18.18% cases of *Plasmodium vivax*. Coexistence of intense fever was observed in 3.33% of *falciparum* and 2.6% of *vivax* malaria.

Plasmodium vivax infection caused by strains with low sensitivity to primaquine are widespread in western pacific and south-east Asia and have recently been reported from central America as well (Signorini *et al.* 1996). A case of primaquine failure in *Plasmodium vivax* infection was recorded in Guatemala. A 28 year old Italian women had *vivax* attack 2 months after returning from Guatemala who was treated there with chloroquine course (1500 mg over 3 days) alongwith primaquine (15 mg/ day) for 14 days. Two months later she had a relapse that was again treated with chloroquine and primaquine at the same dosage. After 3 months a second relapse occurred. This time primaquine (30 mg/ day for 14 days) was administered. The patient remained well during a follow up period of 6 months. Dosage of primaquine as high as 6 mg/kg body weight is recommended in the treatment of *vivax* malaria in central America.

Vivax malaria is most frequent imported malaria in Japan comprising about 60% of the total cases. Patients were usually treated after acute phase therapy of chloroquine with standard course of primaquine (15 mg base daily for 14 days) as curative therapy. Recently however cases of relapse of *vivax* malaria after this standard primaquine therapy are reported from various countries such as Japan, New Guinea, Thailand and Indonesia. In contrast the relapse rates of cases acquired in India are low. Most of the relapsed cases are successfully treated with either of these regimen (i) 20 mg/day for 7 days (ii) two courses of the standard primaquine therapy given one month apart (iii) 15 mg/ day for 21 days without noticeable side effects. It is imperative to establish the most appropriate regimen with primaquine for curative treatment of *vivax* malaria contracted in the areas mentioned above. Smoak *et al.* (1997) studied relapse patterns in US army troops in Somalia. Following initial attack of malaria 60 of 75 cases received a standard course of primaquine (15 mg base daily for 14 days). 26 of 60 soldiers subsequently relapsed with a failure rate of 43%. 8 soldiers had second relapse following primaquine therapy after both primary attack and first relapse. 3 of these soldiers had received a higher dose of primaquine (30 mg base daily for 14 days) after their second attack. The apparent ineffectiveness of primaquine therapy

in preventing relapse suggest the presence of primaquine resistant *Plasmodium vivax* strain in Somalia.

Studies conducted on *Plasmodium vivax* infections indicate that the parasite show tolerance against both therapeutic and antirelapse drugs. A study on relapse / reinfection pattern of *Plasmodium vivax* was conducted in district Shahjahanpur by Prasad *et al.* (1991) who reported maximum number of relapse/ reinfection in a 47 year old male patient, who suffered 8 times. They have indicated that relapse rate was high in males (70.2%) as compared to females (29.8%). A study on relapse pattern of *Plasmodium vivax* was conducted by Sharma *et al.* (1990) in Kheda district of Gujrat where relapsing tendency in *Plasmodium vivax* was observed within 8 months of primary attack. Relapse rate was 2.6% within one year in patients treated with 5 days course of primaquine. Immune response of *Plasmodium vivax* in a cross-section of the population in Delhi area was studied by Ray *et al.* (1994). They observed antibodies against crude blood stage antigen in majority of the individuals who were acutely infected with *Plasmodium vivax*. Studies on *Plasmodium vivax* relapse pattern was conducted in Kheda hospital by Srivastava *et al.* (1996). They treated one group of patients with 600 mg chloroquine and other with 600 mg chloroquine and 50 mg pyrimethamine which yielded 28.3% and

27.3% relapse rates, respectively. They have also noticed more short-term relapses (within 2-3 months) and a few long-term relapse. A five-year epidemiological study of patients attending a malaria clinic in Delhi was conducted by Adak *et al.* (1998) to find out the relapse rate in *Plasmodium vivax* infections. They have also observed with seasonal correlation between the primary infection and subsequent relapses, the duration of the incubation period, and the pattern of relapse. They recorded relapse rate between 23% to 44% depending on the short and long durations of the follow up.

It has also been reported recently that *Plasmodium vivax* has developed resistance to chloroquine. Baird *et al.* (1997) noticed chloroquine resistant strain of *Plasmodium vivax* in Indonesia, 21 patients infected with *Plasmodium vivax* were given chloroquine. 3 of them had recurrent asexual *Plasmodium vivax* parasitaemia between day 14 to 18 despite effective levels of chloroquine at the time of recurrence. Resistance to standard chloroquine therapy by *Plasmodium vivax* was noticed in 14% cases. Marlerthan *et al.* (1995) noticed resistance to standard chloroquine therapy by *Plasmodium vivax* infections, in Myanmar. 50 patients with *Plasmodium vivax* infection were treated with standard regimen of chloroquine phosphate (1500 mg over 3 days) followed by 45 mg primaquine immediately and then weekly for 8 weeks. 43 patients

showed recrudescence between day 3 and 14 with RI, RII and RIII pattern in 1, 3 and 3 patients, respectively. All the chloroquine resistant cases were again treated with 1500 mg chloroquine and no further recrudescence or relapse were detected. In February 1999 in India KEM Hospital in Mumbai recorded first case of *vivax* malaria showing resistance against routine curative drugs.

MATERIALS AND METHODS

Present study is based on the malaria cases enrolled in Medical college and a few other hospitals and clinics of Aligarh during years 1998 and 1999. Blood smears were prepared from patients who attended hospitals and clinics, complained for fever and headache and were suspected for malaria. Thick and thin blood smears of patients were prepared by fingerprick, stained with Jaswant Singh and Bhattacharya and Giemsa stains and microscopically examined under an oil immersion lens to see the positively for malaria infection.

Preparation and fixation of blood smear

Good quality clean glass slides of about 1.2 mm thickness were used for making smears. Greasy sides were first cleaned by boiling with detergents, subsequently scrubbed and washed thoroughly in running water so as to remove all traces of detergents and grease. For making blood smear the tip of middle or ring finger of the left hand was cleaned with spirit swab and allowed to dry completely before pricking. Two drops of blood were collected one for thick smear and the other for thin. The thick smear was made in

such a way that the edge of smear remain approximately 1.0 cm away from the edge of the slide. The distance between the edge of thick and thin smear was about 0.75 cm. Only thin smear were fixed with methyl alcohol. After drying, slides were stained with Giemsa and JSB stains as described by Singh (1956).

Preparation of staining solutions

(i) Preparation of JSB-1 solution:

For preparation of JSB-1 solution 0.5 mg of methylene blue was dissolved thoroughly in 500 ml distilled water in flask. 3 ml of one percent sulphuric acid was added drop by drop to the solution and then mixed with 0.5 gm of potassium dichromate. Addition of potassium dichromate led to the formation of some precipitate and at this step 3.5 gm of disodium hydrogen phosphate dihydrate was added. The mixture was boiled for half an hour. The stain was allowed to cool at room temperature and was ready for use as JSB-1 solution. This solution when used after maturation of 2-3 weeks gave better results.

(ii) Preparation of JSB-II solution

It was prepared by dissolving 1 gm of water-soluble eosine in 500 ml of distilled water. A freshly prepared eosine solution,

although can be used immediately, may not yield as satisfactory result as the one which has turned deep red after some storage.

(iii) Preparation of buffer water

This was prepared by dissolving disodium hydrogen phosphate dihydrate (0.22 gm) and potassium acid phosphate (0.74 gm) in one litre distilled water. To maintain initial pH buffered water was kept in glass bottles.

Staining

Slide staining was done with JSB in following steps:

(a) 2-3 dips in JSB-II

(b) Washing ten times with buffer water

(c) A dip of 45 seconds in JSB-1

(d) Washing ten times with buffer water

(e) Drying thoroughly before examination

A good number of slides were also stained with Giemsa. For staining with Giemsa, smears were first fixed in methanol and dried in air by waving hand. Dried slides were transferred in Giemsa stain for 3-5 minutes then passed on to the same stain diluted with

distilled water (1:1) and allowed it to stain for 15-20 mts. Finally slides were washed thoroughly under tap water and dried.

Examination of slides

Slides were examined under the microscope with 10 x and 40 x objectives to see the positivity for malaria parasite. Positive slides were then thoroughly examined under an oil immersion lens (100 x) to ascertain the type of infection (i.e. *Plasmodium vivax*, *Plasmodium falciparum* or mixed type).

From malaria positive cases monthly *Plasmodium vivax* and *Plasmodium falciparum* infections and their percentage in adults and children was worked out. Month-wise slide positivity rates (SPR) and slide *falciparum* rates (SFR) were also worked out for the years 1998 and 1999. Resistant and relapse cases were also recorded in *Plasmodium falciparum* and *Plasmodium vivax* infections, respectively.

Drug resistance in *Plasmodium falciparum* infections

For drug resistance, patients who were positive for *Plasmodium falciparum* infection were given 1500 mg chloroquine base in divided doses (i.e. 600 mg on day 0, 300 mg after 8 hours

followed by 300 mg daily for 2 days). The dose of child was adjusted accordingly. Blood films of those patients who reported back with fever within 4 weeks of treatment were examined for the *Plasmodium falciparum* infection. If found positive such cases were recorded as chloroquine resistant cases. Level of resistance (i.e. RI, RII, RIII) was ascertained on the basis of late and early recrudescence.

Relapse in *Plasmodium vivax* infections

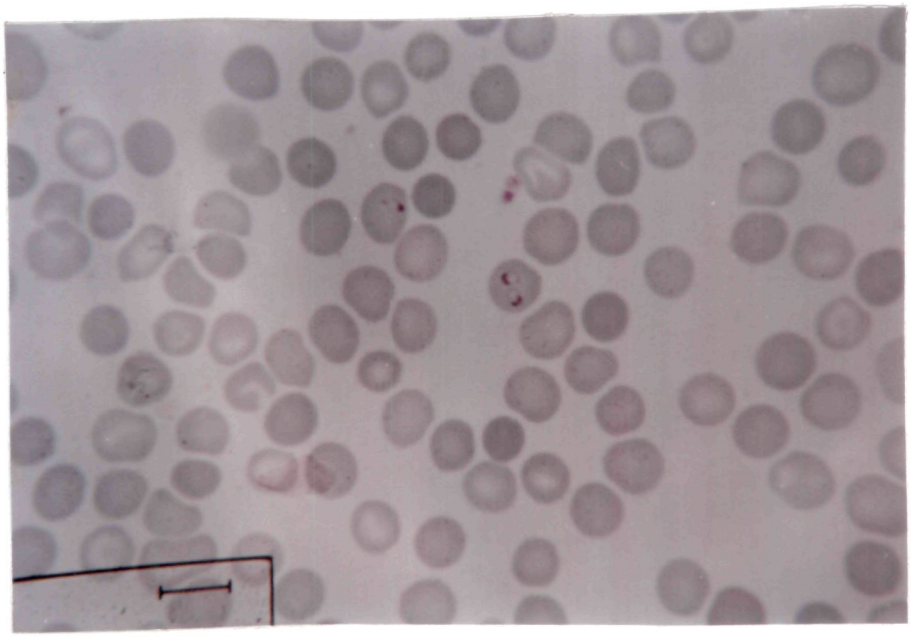
For the study of relapse in *Plasmodium vivax*, one group of patients was treated by giving 1500 mg chloroquine base in divided doses (i.e. 600 mg on day 0, 300 mg after 8 hours followed by 300 mg daily for 2 days). The dose of child was adjusted accordingly. While patients in other group were administered 1500 mg chloroquine in the similar manner followed by 15 mg primaquine daily for 5 days and then followed up carefully. To determine the pattern of relapse in *Plasmodium vivax* each patient was identified individually by name, address and subsequent treatment. On reporting back blood smears were prepared from the patient and examined microscopically for the presence of malaria parasite and entered against his/ her name.

The following criteria were used in classifying the patients into primary cases and non-relapse and relapse categories in the present study. Patient reporting for the first time (having no history of malaria) with acute illness and showing symptoms such as high fever, severe headache, loss of appetite, occasional vomiting and microscopic evidence of *Plasmodium vivax* infection were considered as primary cases. Some patients in this group who had no clinical symptoms of malaria or parasitological evidence of *Plasmodium vivax* infection following their primary infection during the entire study period were considered as non-relapse cases. Those patients who reported back to the clinic within one month to one year with renewed clinical symptom (mild) along with a periodic alternate day fever (not observed in the primary cases) and found to be microscopically positive for *Plasmodium vivax* infection were considered as relapse cases. After medication if patients again suffered from malaria within 3 months with more regular paroxysm he/she was treated as a case of short-term relapse. But if it happened beyond 3 months then the case was considered as a long-term relapse. Cases of *Plasmodium vivax* who did not respond to 1500 mg of chloroquine and 75 mg primaquine were recorded as chloroquine resistant cases.

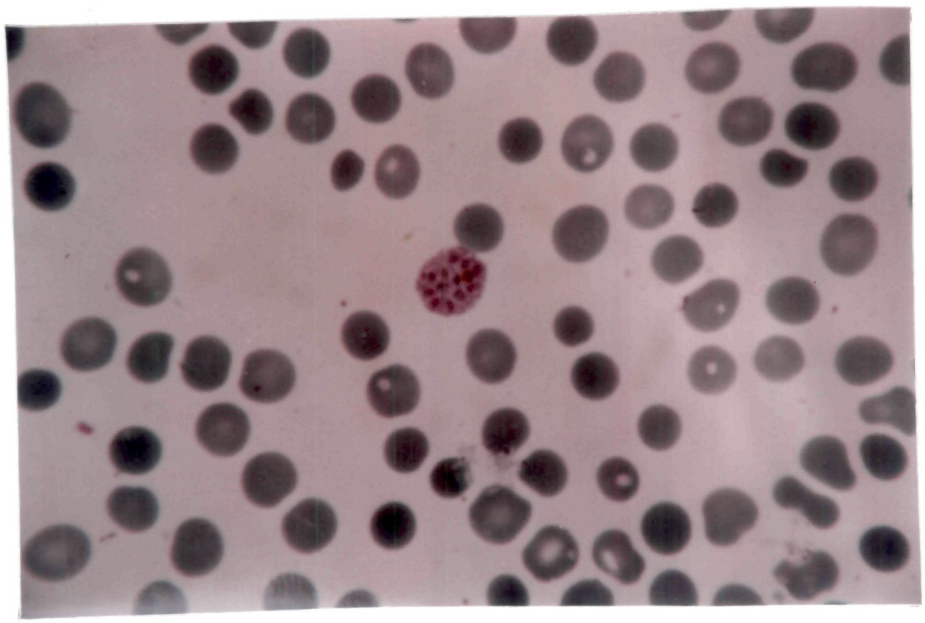
RESULTS

Table I and II show month and year data on *Plasmodium vivax* and *Plasmodium falciparum* cases in the present study. These tables also provide information on seasonality. *Plasmodium vivax* infection was predominant and was recorded in all months of the year with almsost similar seasonal pattern during the two-year study period. *Plasmodium vivax* show a gradual increasing trend from May onwards reaching a peak in September soon after the rainy season and then decline sharply to very low level in December. *Plasmodium falciparum* infections started appearing in August and showed peak in September and October and decreased sharply with the onset of winter (Graph I and II).

During 1998 out of 1256 slides examined, 391 were found positive for malaria of which 272 belonged to *Plasmodium vivax* and 119 to *Plasmodium falciparum* (Table-I). In 1999 a total of 1526 slides were examined, out of which 496 were positive for malaria. Slides showing positivity for *Plasmodium vivax* and *Plasmodium falciparum* were 307 and 187, respectively (Table-II). During years 1998 and 1999 overall percentage of *Plasmodium vivax* and *Plasmodium falciparum* infections were 49.56 and 62.2 and 30.43



Signet ring stage of *P. vivax*

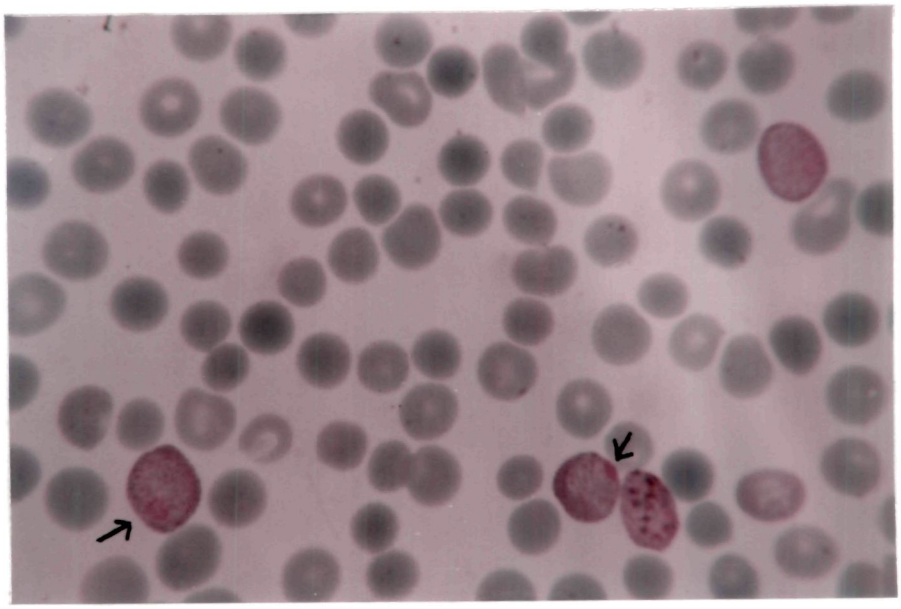


Schizont stage of *P. vivax*

and 37.8, respectively. During these years infection rates in adults and children ranged between 62.2 to 64.7% and 35.2 to 37.7% respectively (Graph III and IV).

It was observed that malaria transmission was least from January to April. During the months of May, June and July transmission was low with slight fluctuating figures for the years 1998 and 1999 which was in accordance with the commencement of pre-monsoon shower that contributed slightly increased or decreased transmission rate in proceeding months. Increased rate of transmission was recorded July onwards, reaching a peak soon after rains in September and October followed by a sharp decline to a low level in November and December. During peak transmission season mean temperature and relative humidity ranged somewhere around 26 to 28°C and 77 to 88%, while in seasons showing low transmission rate, temperature was either too low around 12 to 15°C or too high around 26 to 34°C which was probably the main factor for low malaria transmission (Graph V and VI).

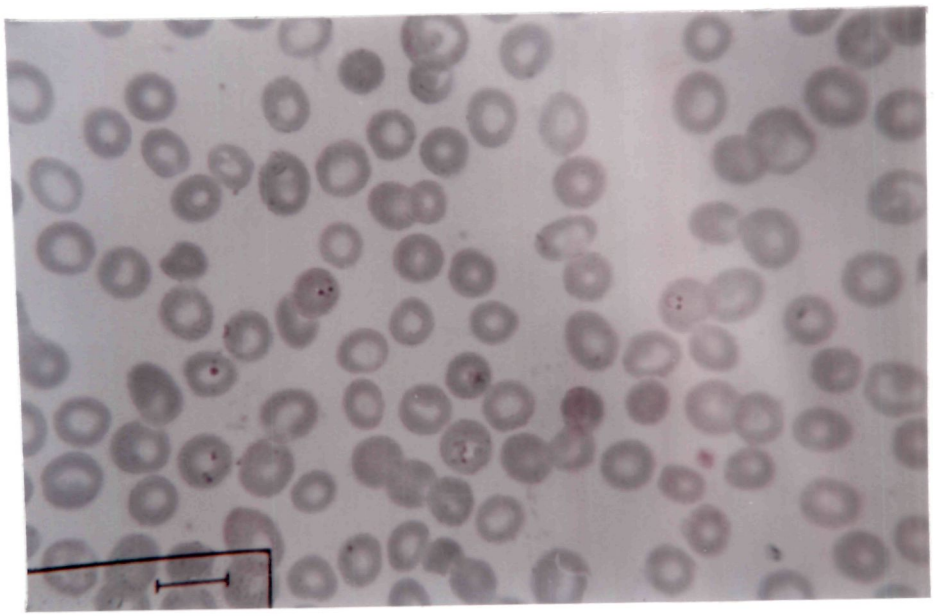
Plasmodium vivax and *Plasmodium falciparum* showed almost similar transmission pattern during the years 1998 and 1999 having mean SPR 31.18 and 30.78, respectively. A considerable degree of fluctuation was observed during peak transmission season (i.e. September and October) when SPR was comparatively high and



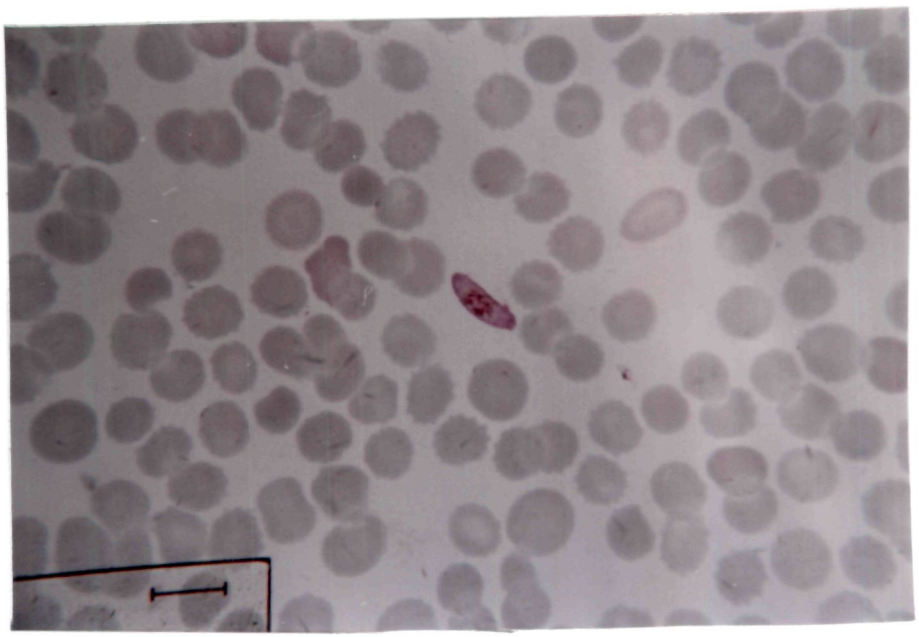
Gametocyte of *P. vivax*

ranged between 36.3 and 44.6% (Table-I and II). *Plasmodium falciparum* infection was also proportionately high showing slide *falciparum* rate between 12.9 and 16% during these months for both the years followed by a low rate (i.e. 5.7 to 8.08%) during winters and lowest in summer which ranged from 2.3 to 5.1%.

Table III shows yearly analysis of P. vivax, non-relapse versus relapse patients and the relapse rate percentage with different follow up durations. In 1998, the total number of *Plasmodium vivax* patient was 150. Of which 126 did not have any further relapse, whereas 24 had relapses in two year follow up study, giving a relapse rate of 16%. Similarly for year1999 the relapse rate calculated was 11.3%. Short-term relapses were more then the long-term relapses in the ratio of about 3:1 for both the years. In 1998 and 1999 short and long-term relapses were recorded as 12 and 4% and 7.8% and 3.4%, respectively. Above percentage of relapse was recorded in patients who were given 1500 mg chloroquine in divided doses in adults (600 mg on day 0, 300 mg after 8 hours followed by 300 mg daily for two days). 65 patients who were administered with 1500 mg chloroquine and 75 mg primaquine in divided doses also showed relapses in 3.07% cases. During entire study period three *Plasmodium vivax* cases showed resistance against chloroquine as the parasite showed recrudescence on 9^{th}, 11^{th} and 14^{th} day after



Signet ring stage of *P. falciparum*



Gametocyte of *P. falciparum*

the administration of standard dose of 1500 mg chloroquine and 75 mg of primaquine.

Table IV shows the frequency distributions of 80 and 140 patients, showing resistance against chloroquine in 7.5 and 10% cases after getting 1500 mg chloroquine base in divided doses (600 mg on day 0, 300 mg after 8 hours followed by 300 mg daily for two days). *Plasmodium falciparum* primary attach recorded in individuals who did not have any previous malaria history during non-transmission months, particularly from December to June in order to rule out possibility of recrudescence because of incomplete medication. Of 80 patients studied during year 1998 seventy four were susceptible whereas 6 patients showed resistance against chloroquine. The level of resistance was RI and RII type in 5 and 2.5% cases as they relapsed around 10^{th} and 5^{th} day after medication. Similarly during year 1999 out of 140 cases studied, 126 were susceptible while 14 showed resistance, out of which 6.4% were of RI type and 3.5% were of RII type.

TABLE-I

Prevalence of malaria showing parasite distribution, SPR and SFR during year1998

Years	BSE	Total cases	P. vivax	P. falciparum	SPR	SFR
Jan	52	15	11	4	28.8	7.6
Feb	57	20	16	4	35	7.0
Mar	39	12	10	2	30.7	5.1
Apr	55	12	10	2	21.8	3.6
May	43	13	10	3	30.2	6.9
Jun	72	25	20	5	34.7	6.9
Jul	98	15	12	3	15.3	3.06
Aug	120	24	16	8	20	6.6
Sep	280	125	80	45	44.6	16
Oct	220	80	50	30	36.3	13.6
Nov	140	30	22	8	21.4	5.7
Dec	80	20	15	5	25	6.2

TABLE-II

Prevalence of malaria showing parasite distribution, SPR and SFR during year1999

Months	BSE	Total cases	P. vivax	P. falciparum	SPR	SFR
Jan	43	13	10	3	30.2	6.9
Feb	23	10	8	2	43.5	8.7
Mar	25	8	7	1	32	4
Apr	42	6	5	1	14.2	2.3
May	33	15	12	3	45.6	9.09
Jun	48	11	9	2	22.91	4.16
Jul	75	20	16	4	26.6	5.33
Aug	156	32	26	6	20.5	3.8
Sep	482	191	129	62	39.6	12.9
Oct	350	133	85	48	38	13.71
Nov	150	39	22	17	9.8	4.2
Dec	99	19	11	8	19.1	8.08

TABLE-III

Relapse cases in *Plasmodium vivax* infection

Year	Total no. of P. vivax patients observed	Non-relapsing patients	Relapsing patients	Short-term relapse	Long-term relapse	Relapse rate	Follow up years
1998	150	126	24	18	6	16	2
1999	115	102	13	9	4	11.3	1
1999	65*	63	2	2	0	3.07	0.5

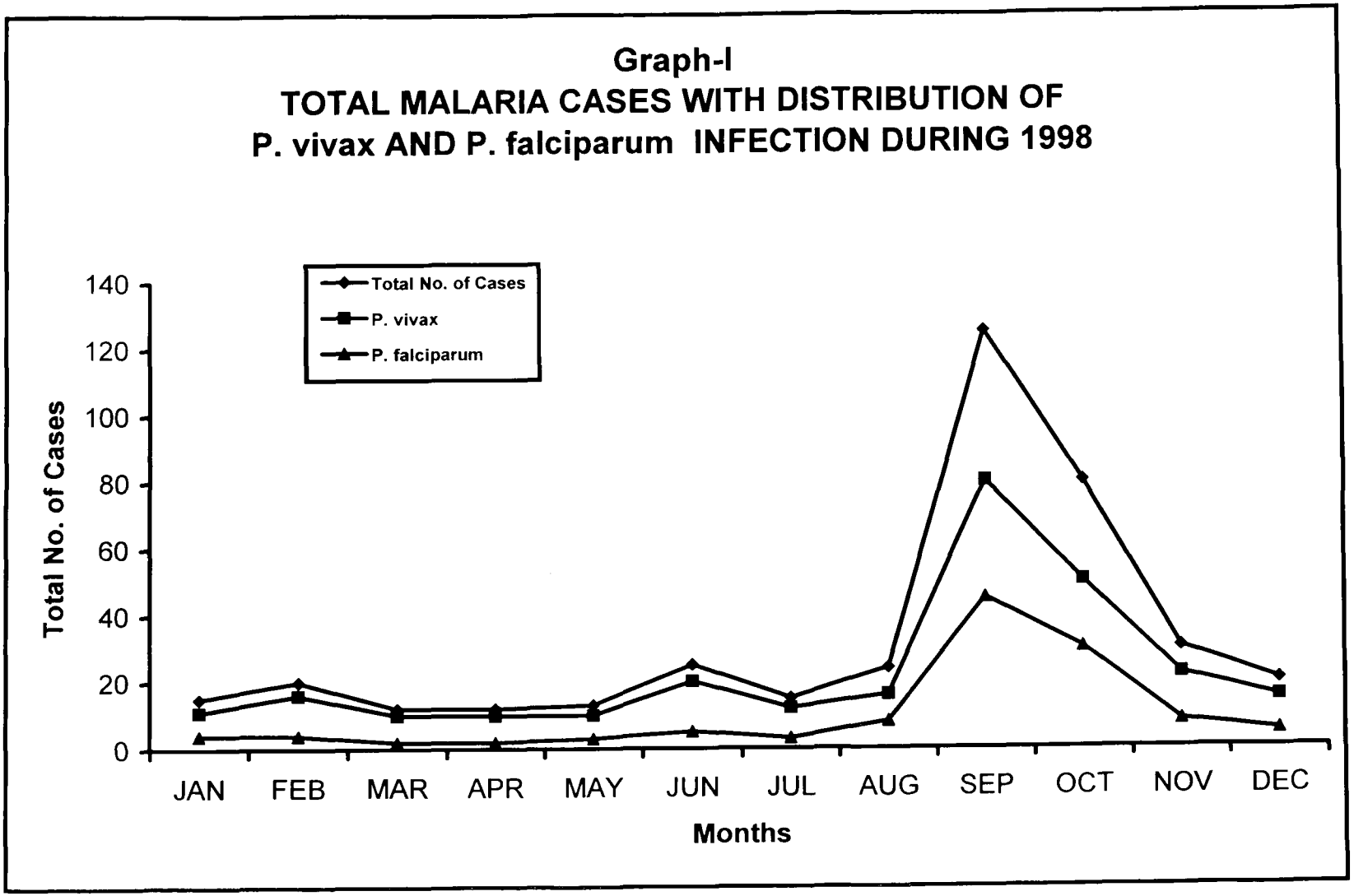
*** Patients given 75 mg primaquine base following chloroquine treatment**

TABLE-IV

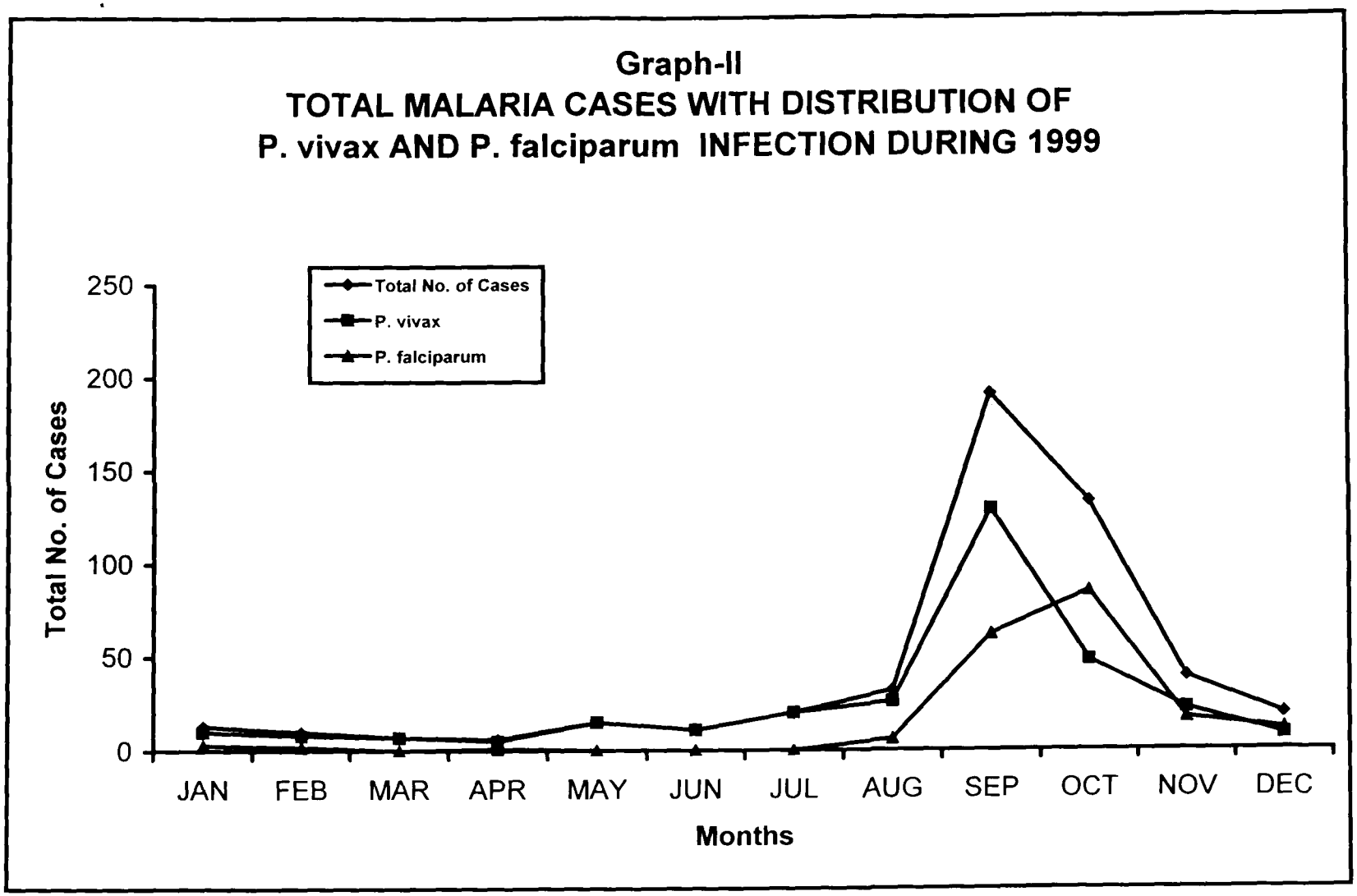
Chloroquine resistant cases in *Plasmodium falciparum* infection

Months	Total no. of P. falciparum patients observed	Non-Resistant patients	Resistant patients				Resistance rate			
			Total	RI	RII	RIII	Total	RI	RII	RIII
1998	80	74	6	4	2	-	7.5	5	2.5	-
1999	140	126	14	9	5	-	10	6.4	3.6	-

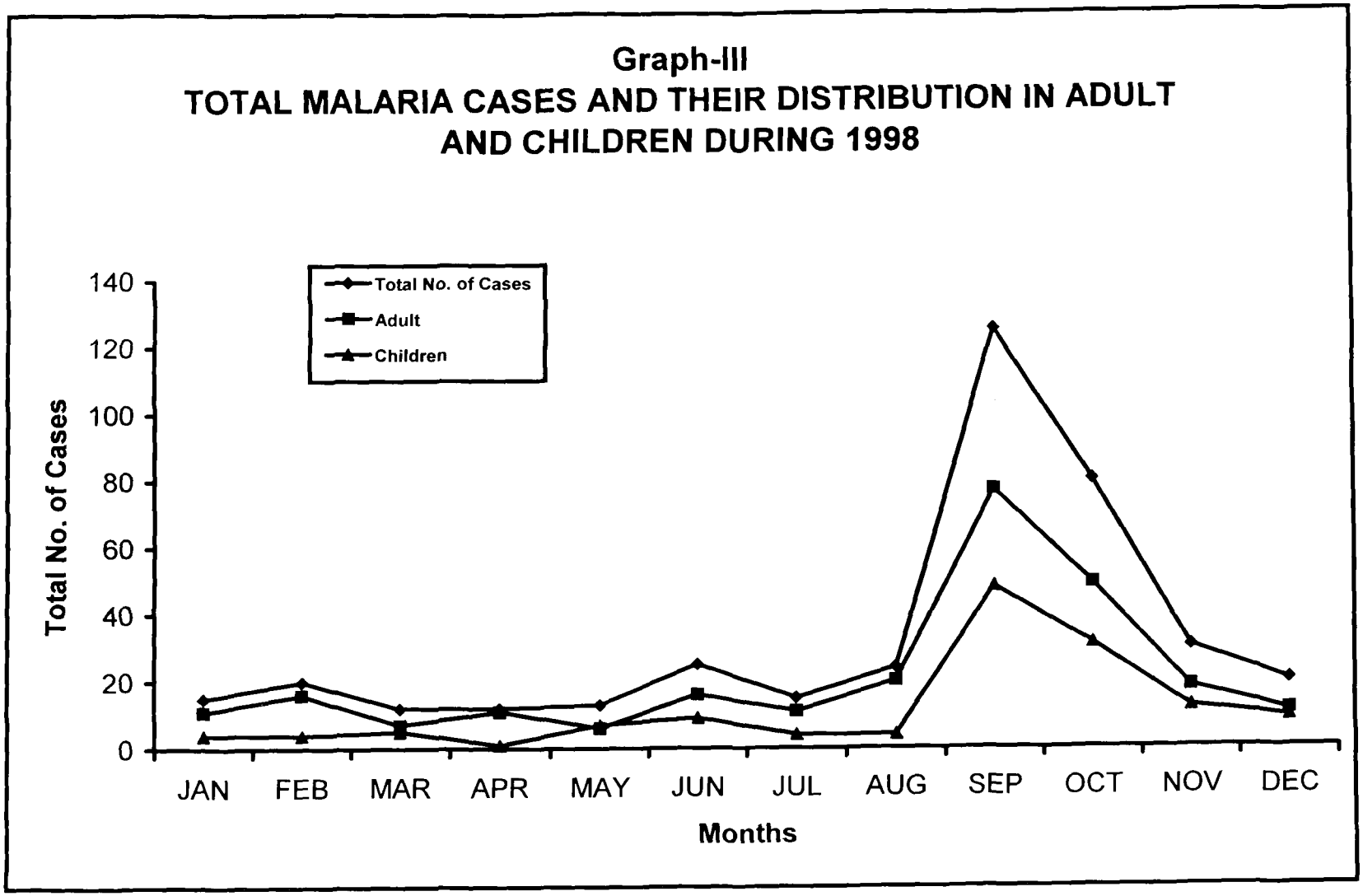
Graph-I
TOTAL MALARIA CASES WITH DISTRIBUTION OF P. vivax AND P. falciparum INFECTION DURING 1998



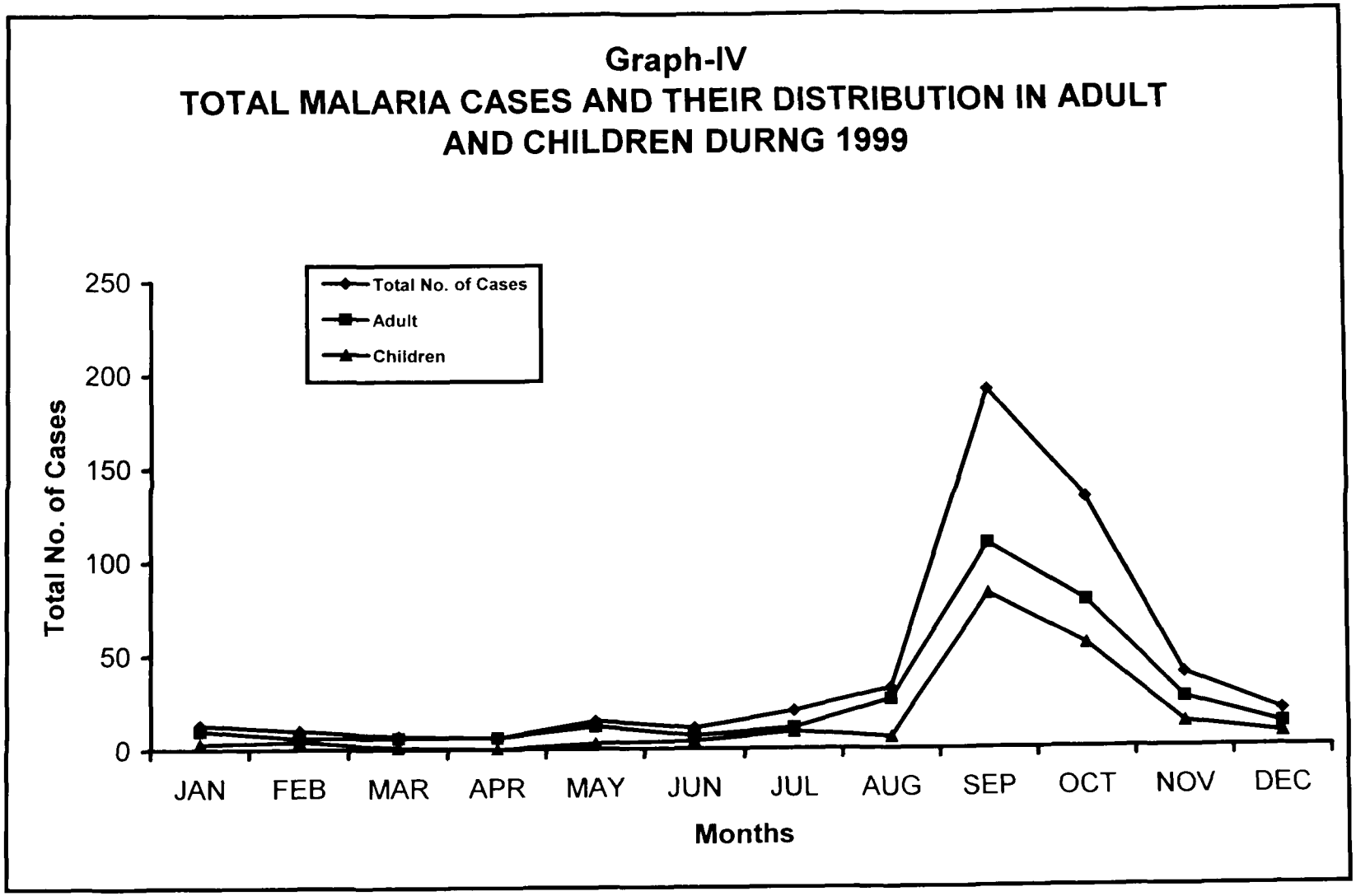
Graph-II
TOTAL MALARIA CASES WITH DISTRIBUTION OF P. vivax AND P. falciparum INFECTION DURING 1999



Graph-III
TOTAL MALARIA CASES AND THEIR DISTRIBUTION IN ADULT AND CHILDREN DURING 1998

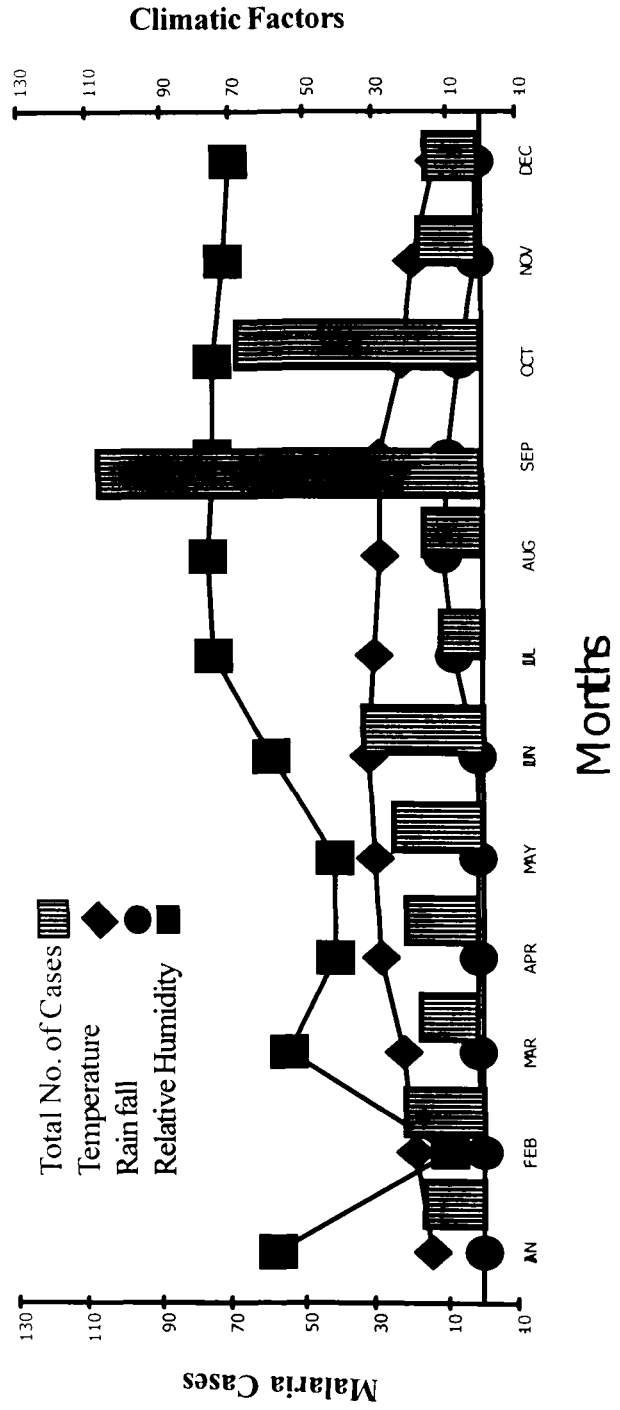


Graph-IV
TOTAL MALARIA CASES AND THEIR DISTRIBUTION IN ADULT AND CHILDREN DURNG 1999



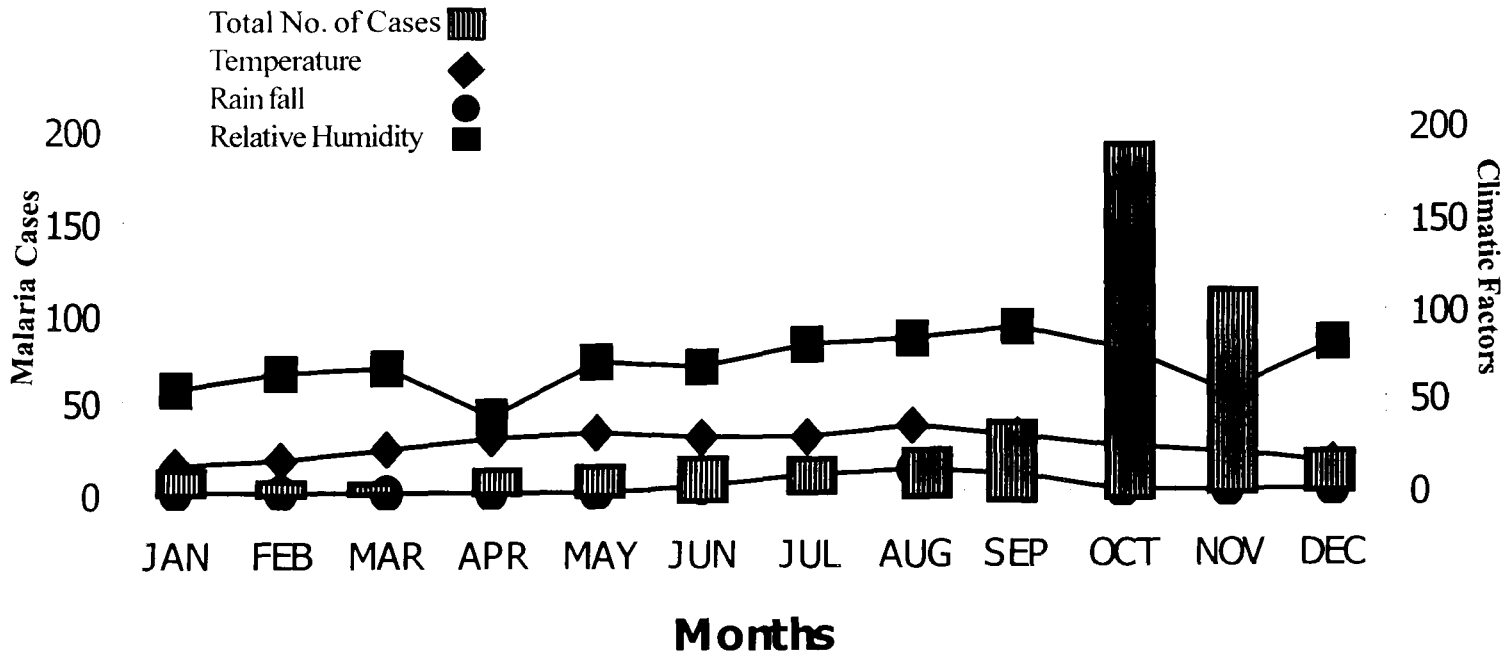
Graph-V

TOTAL NO. OF MALARIA CASES VS CLIMATIC FACTORS DURING 1998



Graph-VI

TOTAL NO. OF MALARIA CASES VS CLIMATIC FACTORS DURING 1999



DISCUSSION

Malaria still continues to be the most important vector-borne disease causing extensive morbidity and mortality in the tropics. Malaria parasites belonging to genus *Plasmodium* are characterised by heterogeneous life cycle involving a sexual and sporogonic mode of reproduction in the invertebrate host and an asexual reproductive mode involving schizogonic cycles in the vertebrate host.

In the present investigations, patients attending OPD of Jawaharlal Nehru Medical College and a few other clinics of Aligarh showing symptoms such as high fever, severe headache, loss of appetite, occasional vomiting and microscopic evidence of *Plasmodium vivax* and *Plasmodium falciparum* were considered. Resistant and relapsing cases were observed in *Plasmodium falciparum* and *Plasmodium vivax* infections, respectively.

In the present study peak transmission of malaria during the months of September and October which was recorded during year 1998 and 1999 was probably because of the availability of plenty of breading sites for the vector species after rains in proceeding months. Moderate temperature (i.e. 26 to 28°C) and optimum humidity (i.e. 77 to 88%) during these months which were ideal for mosquito breeding might have influenced increase in vector

population which must have contributed maximum transmission as earlier observed by Adak *et al.* 1998 in Delhi. Increased transmission of malaria was also recorded in Assam in areas having streams where vector species *An. minimus* was abundant (Wajihullah *et al.* 1992 and Jana-kara *et al.* 1995). Whereas least transmission which was recorded from December to June was because of adverse environmental factors which caused reduction in number of vector species. During these months either extreme dry cold or dry heat conditions prevail which affect vectors adversely either by making water bodies unsuitable or very slow for breeding or by making them scarce as a result of high temperature which even kills the vector.

Percentage for *Plasmodium vivax* during years 1998 and 1999 was 69.56 and 62.2 while for *Plasmodium falciparum* it was 30.4 and 37.8 indicates a slight decrease in *Plasmodium vivax* and an increase in *Plasmodium falciparum* infection, if 3:1 ratio (i.e. 75% and 25%) is treated as usual one in plains for *Plasmodium vivax* and *Plasmodium falciparum* infections. Development of resistance against routine curative drug (i.e. chloroquine) might have been one of the reasons for this increase in *Plasmodium falciparum* infection. As for higher percentage of infection in adults as compared to children is concerned, it might be because of the reason that adults

are much more exposed to mosquito bite as compared to children who are protected by their parents to some extent. Slide *falciparum* rate (SFR) was considerably high during peak transmission period as well as in colder months even when transmission rate is low. It is difficult to draw exact conclusion but it seems that development of *Plasmodium falciparum* is supported by the vector species more readily as compared to *Plasmodium vivax,* may be it is a particular strain of the same vector species which emerges in good number in winters and contributed for raised *falciparum* transmission. Another probable explanation may be this, that complicated cases generally referred to medical college and a few of them happens to be of *falciparum* malaria, might have contributed to raised number of cases. And since we collected maximum cases from medical college, SFR obtained in the present study is a bit high. In contrast *Plasmodium vivax* cases are generally treated outside easily cured and therefore are not on record, hence its transmission rate, which is observed is proportionately low.

In present study 7.5 and 10% patients suffering from *falciparum* malaria showed resistance by showing recrudescence around first and second week following chloroquine treatment during 1998 and 1999. Sharma (1999) reported 30% resistant cases in high transmission zone in India. Many other reports are available in India

and abroad which show increasing trend of drug resistance. Ghosh *et al.* (1992) observed 25% chloroquine resistant cases in Orissa Shah *et al.* (1997) reported chloroquine resistant cases in Pakistan and Afghanistan. Mharakurwas *et al.* (1997) reported resistance in as much as 52% patients following chloroquine treatment. Bojang *et al.* (1998) reported failure of chloroquine in Gambian children. In present study less percentage of resistant cases were recorded which might be because of the reason that study was conducted in low transmission region were *Plasmodium falciparum* infection is proportionately less.

A very remarkable observation was made during the present study that 3 patients having *Plasmodium vivax* infection showed resistance against chloroquine. Similar observation regarding resistance of *Plasmodium vivax* against chloroquine was made by Baird *et al.* (1997). In another study Marler Than *et al.* (1995) recorded recrudescence between 3 and 14 days following chloroquine and primaquine treatment which is in corroboration with the present finding where chloroquine and primaquine did not respond in few cases.

Relapse rates in *Plasmodium vivax* during year 1998 and 1999 following administration of 1500 mg chloroquine base were 16% and 11.3%, respectively. Short-term relapses were more (12 and 7.82%)

as compared to long term relapses, which were 4 and 3.4%. But relapse rate was quite low (i.e. 3.07%) and was of short-term type, when 75 mg primaquine base was also administered following chloroquine treatment. This indicates that both chloroquine and primaquine were not fully effective even when they were administered in a total curative dose to the patients suffering from *vivax* malaria. Similar observations were made by Srivastava *et al.* (1996) who recorded more short-term relapses and a few long-term relapse after administration of 600 mg chloroquine, is in accordance with our finding where more short-term relapses were recorded. Adak *et al.* (1998) recorded 23-44% relapsing cases within a period of 5 years study after giving 900 mg chloroquine in 2 doses (600 mg + 300 mg). Smoak (1997) observed relapses in 43% Somalian soldiers who received standard dose of primaquine. In these studies relapse rates were high as compared to our findings. It may be because of the reason that anti-relapse drug, primaquine was not administered by Adak *et al.* while in case of Smoak's study, may be strain of *Plasmodium vivax* was more tolerant to anti-relapse drug, primaquine and did not respond to it in a fairly good number of cases.

Regarding interpretation of the results, one may disagree with the differentiation of primary attack verses relapse or reinfection,

particularly during peak transmission season. However, in the absence of any clinical or parasitologic marker, the following observations are considered as relevant. In the present study, malaria cases detected between December and June (the supposed non-transmission season) could be grouped in three categories:

(1) Infections acquired in the previous transmission season i.e., between July and November but remained undetected and thus untreated. (2) Infections acquired during the previous transmission season that were detected, treated and subsequently reappeared (relapse); or (3) Infections acquired during the previous transmission season that became clinically and parasitologically positive after a prolonged period (delayed primary attack).

Although the reinfection particularly during the main transmission season could not be ruled out, real transmission from December to June is probably occasional and at a very low level, if it occurs. Therefore the majority of cases detected during the supposed non-transmission period with a definite history of malaria were considered to be relapse rather than reinfections, whereas those detected with a history of malaria were considered delayed primary attacks. However, some uncertainty still exists regarding the possibility of reinfiection during the supposed non transmission season, which probably will persist until some diagnostic tool is

developed that can distinguish the primary attack and subsequent relapse from reinfection.

It is quite evident from the duration of follow up study (1-2 years) that the average resistance rate for the year 1998 was 7.5% and for the year 1999 (10%). The relapse rate for the year 1998 and 1999 were 16% and 11.3% respectively. Therefore the present drug policy of the National Malaria Eradication Programme for the administration of primaquine (15 mg of base, once a day for five consecutive days) for radical cure of *Plasmodium vivax* infection warrants its reconsideration.

The mechanism of long survival of *Plasmodium* vivax during non-transmission months through primary long incubation and relapses actually ensures transmission in the next season through the existence of a reservoir of *Plasmodium vivax*. It is assumed that the primary long and late relapse mechanism of *Plasmodium vivax* might have evolved to survive into the next transmission season and be dormant to avoid the host immune response.

In view of this information, it is suggested that the frequency distribution/ratios of different parasite forms responsible for different relapse patterns should be determined in different *Plasmodium vivax* ecosystems with reference to space and time, which are probably not constant and likely to be time dependent. In addition, the degree

to which these parasite sub populations interact with each other will no doubt have an impact on the maintenance of genetic diversity and regulation of the parasite population as a whole. However, in the absence of parasitologic and clinical markers, it may be difficult to characterise these forms, perhaps amplification of specific DNA sequences by the polymerase chain reaction using specific oligonucleotide probes from different parasite isolates of relapsing and non-relapsing patients could be used to analyse the genetic diversity of the *Plasmodium vivax* population and correlate this with epidemiological findings. Therefore there is a strong need for integrated laboratory and field studies as well as the use of mathematical models to interpret the complex transmission dynamics of *Plasmodium vivax* and *Plasmodium falciparum* so that appropriate malaria control strategies, including chemotherapeutic measures, can be devised.

In view of the above findings it become evident that in Aligarh both resistant and relapse cases are coming up in *Plasmodium falciparum* and *Plasmodium vivax* infections and it is need of the time that at least in cases showing resistance, either increased doses of chloroquine must be administered or a revised drug policy should be introduced by Malaria Control Programme so that alternative drugs should be given for the treatment of malaria patients especially those suffering from *Plasmodium falciparum* infection.

SUMMARY

Two years study of patients attending Jawaharlal Nehru Medical College and a few other clinics of Aligarh was conducted to find out the percentage of *Plasmodium vivax* and *Plasmodium falciparum* infections. Seasonal correlation of these infections was also studied. Relapse and resistance rates were determined in *Plasmodium vivax* and *Plasmodium falciparum* infections. *Plasmodium vivax* and *Plasmodium falciparum* infections during 1998 and 1999 were 69.59% and 62.2% and 30.43 and 37.8%, respectively. Peak transmission of malaria with a slide positivity rate of 38 to 44.6% and slide falciparum rate of 12.9 to 16% was recorded during September and October, which happened to be the peak transmission months. During these months mean temperature ranged around 26 to 28°C while relative humidity was 77 to 88%. Lowest transmission was recorded during March and April when slide falciparum rate was minimum (2.3 to 5.1%). Relapse rates recorded during year 1998 and 1999 were 11.3 and 16% in patients received 1500 mg chloroquine. About 7.5% of relapsing cases were of short-term type. Patients who received 1500 mg chloroquine and 75 mg primaquine also relapsed but frequency was less (3.07%).

Three chloroquine resistant cases of *Plasmodium vivax* were also recorded in the present study. 75 and 10% patients suffering from falciparum malaria during year 1998 and 1999 showed resistance against chloroquine. Level of resistance in these cases was of RI and RII types.

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